THE EFFECTS OF CERTAIN ANTITHYROID DRUGS ON THE UPTAKE OF RADIOACTIVE IODINE BY THE FROG THYROID [1]

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Within recent years a number of chemical substances which inhibit the activity of the thyroid gland have been extensively studied by Astwood (1943), MacKenzie and MacKenzie (1943), Astwood, Bissell and Hughes (1945), McGinty and Bywater (1945), VanderLaan and Bissell (1946a). In general, these antithyroid substances fall into three classes: (1) thiourea and its derivatives, (2) aniline derivatives, including the sulfonamides and other aminobenzene compounds, (3) thiocyanates.

One method of approach to the problem is the use of radioactive iodine (I^{131}) as a tracer for following the course of injected iodine in animals treated with thyroid-inhibitors. With radioactive iodine tracer techniques, it has been demonstrated by Franklin, Lerner and Chaikoff (1944) and Rawson, Tannheimer and Peacock (1944) that the oral administration of thiouracil to rats and chicks for periods of several days results in a marked reduction in the natural capacity of thyroid tissue to concentrate iodine. In another study, Larson, Keating, Peacock and Rawson (1945) demonstrated that a single injection of 10 mg. of thiouracil markedly inhibited the collection of radioactive iodine by the chick thyroid.

Such studies, for the most part, have dealt with warm-blooded animals. The present paper is a report of experiments carried out on the frog to determine (1) whether frog thyroids treated with thyroid-inhibitors concentrate iodine as effectively as those of control animals; (2) whether there are differences in the action of the three groups of antithyroid compounds; (3) whether there are any significant differences between warm-blooded and cold-blooded forms with respect to the mechanisms involved in the goitrogenic effects of these substances.

Materials and Methods

Male and female frogs (*Rana pipiens, Rana clamitans* and *Rana palustris*) of 50 to 65 grams body weight were kept in a large screened cage under dripping water in an air-conditioned room at 5° C.

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In the first series of experiments, groups of experimental animals were given single sub-cutaneous injections of the drugs to be tested. Those receiving thiourea or sulfanilamide were given 0.5 cc. of a 10 per cent solution; those receiving potassium thiocyanate were given 1 cc. of a 0.2 per cent solution. Twenty-four hours after the drug injection a dose of approximately 22.5 microcuries of carrier-free radioactive iodine in a sodium bisulphite solution was injected intraperitoneally into both experimental and control animals. Uniform groups of animals were sacrificed 20 minutes, 40 minutes, one hour, three hours, 8 hours, 24 hours, and 48 hours after injection of the radioactive iodine. The thyroids were removed, rinsed in frog Ringer's solution, blotted, weighed, pressed flat on clean microscope slides, and radioactivity counts made.

In the second series of experiments the experimental animals were given the same doses of the test compounds as above. At intervals of one hour, three hours, 8 hours, 24 hours, 48 hours, and, in some cases, 72 hours after injection of the test compounds, groups of animals were injected intraperitoneally with 22.5 microcuries of carrier-free radioactive iodine in a solution of sodium bisulphite. The control animals received only the radioactive iodine. The animals were sacrificed three hours after the injection of labeled iodine. The thyroids were removed and prepared for reading as above.

The first and second series of experiments showed that potassium thiocyanate disappeared from the circulation approximately 24 hours after its injection into the animal. For this drug a third series of experiments was performed in order to see if the curve for its effectiveness at successive time intervals was similar to the curves for thiourea and sulfanilamide. In this series of experiments the drug was injected and at one minute, 20 minute, 40 minute, one hour, three hour, 8 hour, and 12 hour intervals, the radioactive iodine was injected into experimentals and controls; all animals were killed three hours after injection of the iodine.

In the three series of experiments the collection of radioactive iodine by the thyroid glands was detected by serial counting at two-minute intervals with a Geiger-Muller counter. In each group of animals the count of radioactive iodine collected by the thyroids of the experimental animals was compared with that collected by the thyroids of the untreated controls and expressed as per cent of the control uptake of radioactive iodine. The count of radioactive iodine collected by the thyroids of the experimental groups divided by that of the control animals determines this per cent of control uptake.

Results

The thyroid glands of the control animals were normally pale and to the naked eye did not appear enlarged. The weights of these glands varied between 0.0008 and 0.0014 gm. and averaged 0.001 gm. Animals treated with thiourea had thyroid glands which were dark red, obviously vascular and enlarged. The weights of the thyroid glands of these animals varied between 0.0014 and 0.0023 gm. and averaged 0.0019 gm. Animals treated with sulfanilamide had thyroid glands which were dark red, vascular and much enlarged. The weights of this group of thyroids averaged 0.0015 gm. and varied between 0.0012 and 0.0018 gm. The thyroid glands of the potassium thiocyanate-treated animals were dark red, quite vascular and enlarged. The weights of the thyroid glands of these animals averaged 0.0012 gm. and varied between 0.0010 and 0.0014 gm. The injection of the drugs into the experimental

animals produced thyroid glands larger than those of the control animals in every instance. The increase in volume of the glands was apparent to the naked eye and seems to have been greater than was the increase in weight.

Effects of thiourea and sulfanilamide

The effect of a single injection of 0.5 cc. of a 10 per cent solution of thiourea upon the uptake of radioactive iodine by the frog thyroid is shown graphically in Figure 1. It will be seen that the curve for the experimental animals follows that

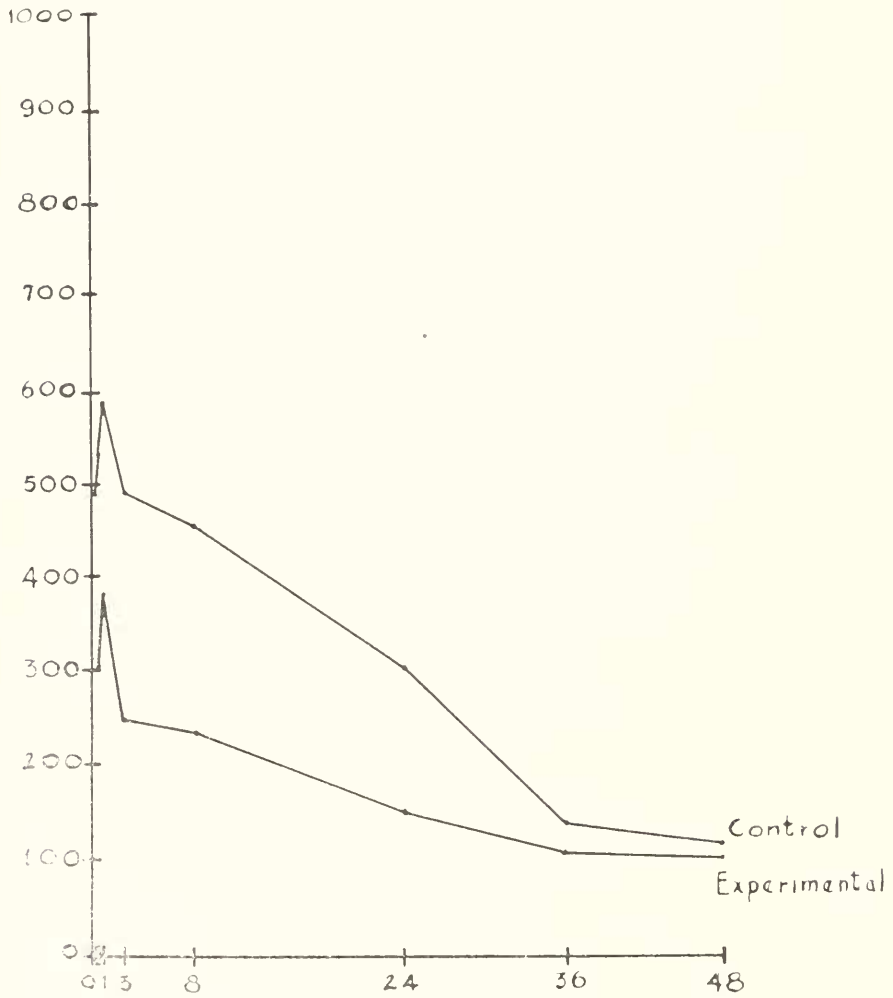

FIGURE 1. The effectiveness of thiourea as an inhibitor of the uptake of I^{131} by the frog thyroid at successive time intervals after injection of the drug. Each point on the graph represents the average of two separate series of experiments. The ordinate shows radioactivity counts per two minutes and the abscissa indicates the time intervals in hours after injection of radioactive iodine.

FIGURE 2. The effectiveness of sulfanilamide as an inhibitor of the uptake of I^{131} by the frog thyroid at successive time intervals after injection of the drug. Each point on the graph represents the average of two separate series of experiments. The ordinate shows radioactivity counts per two-minute intervals, and the abscissa indicates the time intervals in hours after injection of radioactive iodine.

for the controls but is consistently lower. The thiourea-treated animals show an uptake which is 62.0 per cent of that of the controls 20 minutes after the injection of radioactive iodine (24 hours and 20 minutes after administration of thiourea). The percentage falls steadily to a level of 48.5 per cent at the 24 hour period (48 hours after thiourea administration). Then, as would be expected, a gradual recovery is exhibited so that by 72 hours after thiourea administration the iodine uptake of the treated thyroids is approaching that of the controls. It is clearly indicated, therefore, that thiourea does affect the thyroid's ability to take up iodine and that,

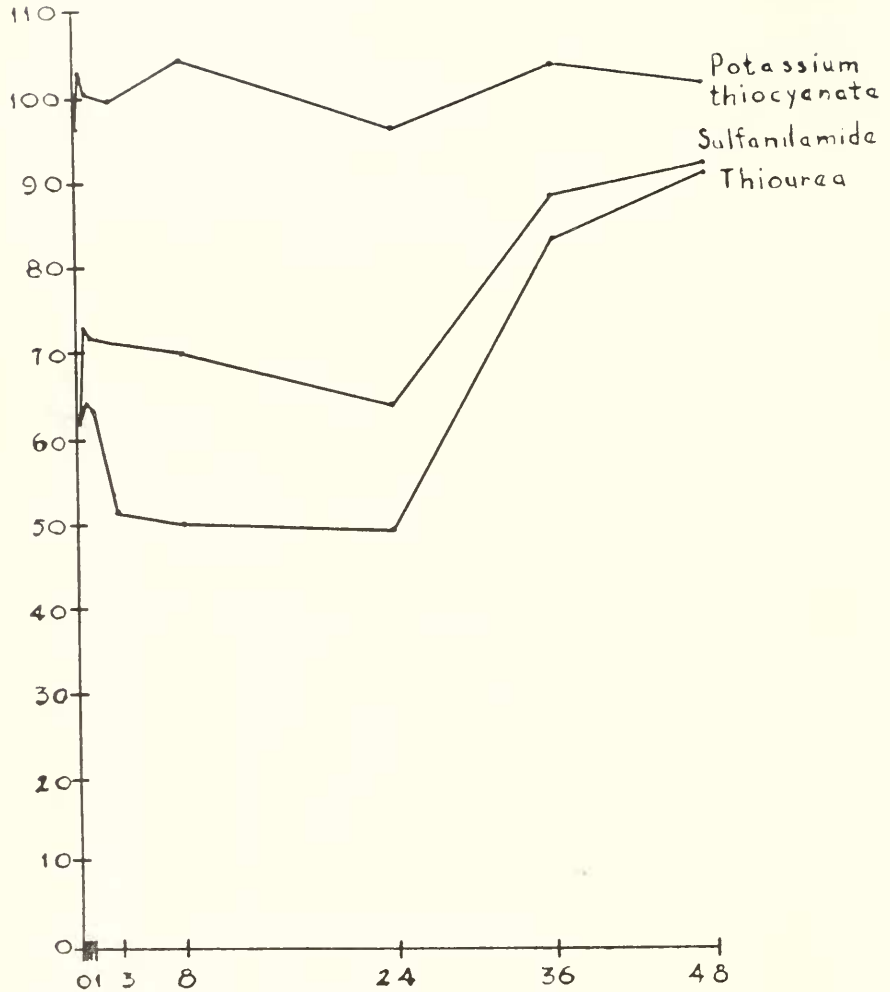

FIGURE 3. The per cent of control uptake of I^{131} by the frog thyroid is compared for thiourea-, sulfanilamide- and potassium thiocyanate-treated animals. Each point on the graph represents the average of two separate series of experiments. The ordinate shows the per cent of the control uptake of I^{131}, and the abscissa indicates the time interval in hours after its injection.

in the frog, a single dose of 0.5 cc. of a 10 per cent solution of thiourea has a maximum effectiveness about 48 hours after the drug is given.

The data for sulfanilamide are graphically presented in Figure 2. The results are clearly similar to those obtained by thiourea treatment, but the effect (at this dosage level) is somewhat less marked, the maximum reduction being only to 63.7 per cent of that of the controls. Another basis for comparison is given in Figure 3 where the curves are based on percentages of control uptake.

After the experiment in which the radioactive iodine was given 24 hours after administration of the thyroid-inhibiting drug and its uptake followed over a 48-hour

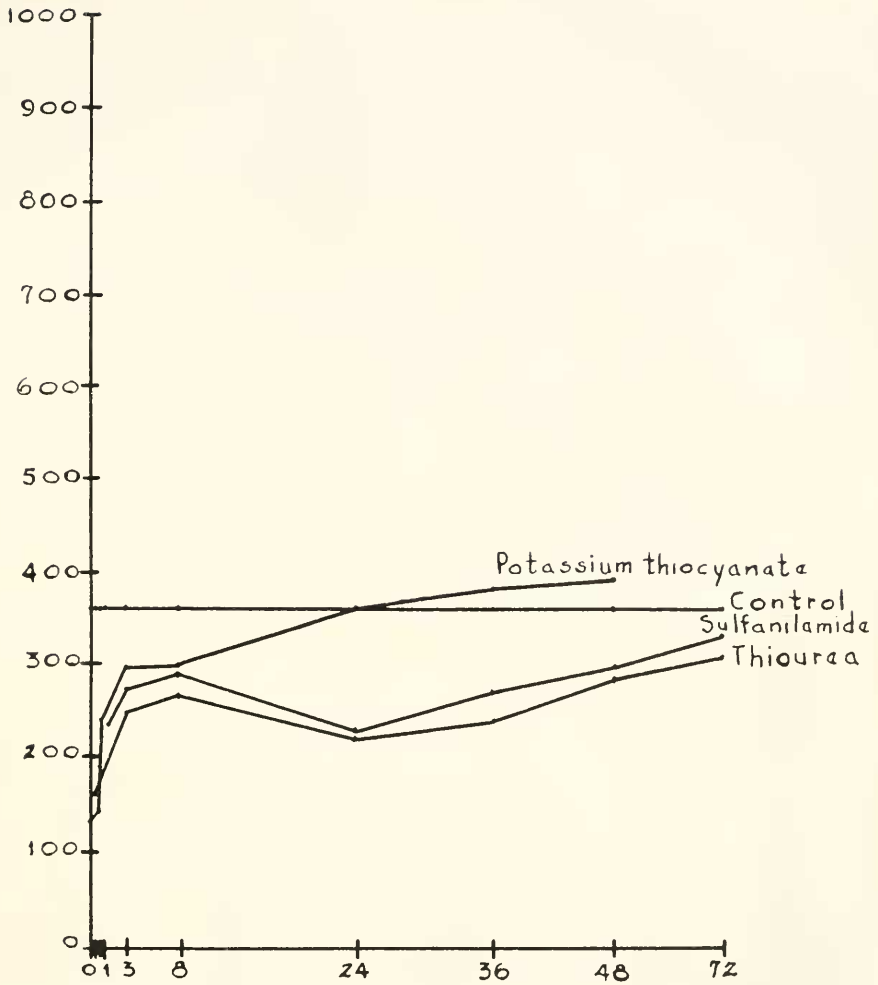

FIGURE 4. The rate of radioactive iodine absorption by frog thyroids treated with thiourea, sulfanilamide and potassium thiocyanate. Each point on the graph represents the average of two separate series of experiments for each drug. The ordinate shows the radioactivity counts per two-minute interval, and the abscissa indicates the time intervals in hours after injection of radioactive iodine.

period, a series of experiments was undertaken in which the radioactive iodine was given at varying times after the drug. The data for thiourea show that the drug effectively blocks iodine absorption almost immediately after its injection. In fact, the maximum effectiveness is seen in the animals which were injected with radioactive iodine only one hour after thiourea injection. With longer intervals the effect is gradually reduced although it is still perceptible after three days. For sulfanilamide the results are similar although again the degree of effectiveness with this dosage is somewhat less than that shown by thiourea.

Effects of potassium thiocyanate

The results of the first series of experiments with potassium thiocyanate showed no significant reduction in iodine uptake by the thyroids of the experimental animals as compared with the uptake by the thyroids of the control animals. The explanation of the failure became apparent, however, when the second series of experiments was carried out. The results of these experiments show that potassium thiocyanate is very effective as an agent for inhibiting iodine uptake by the thyroid, but is rapidly removed from the circulation.

In order to ascertain whether the curve for the effectiveness of potassium thiocyanate at successive time intervals after its injection is similar to the curves for thiourea and sulfanilamide, a third series of experiments was performed in which radioactive iodine was given at intervals from one minute to 48 hours after thiocyanate administration. The results of this study show that the form of the curve for potassium thiocyanate is essentially the same as that for thiourea and sulfanilamide but is displaced to the right; that is, the maximum effect is exerted earlier and the effectiveness declines more quickly. It is to be noted that the uptake of iodine in these thiocyanate-treated animals ultimately reached a level higher than that seen in untreated controls (Fig. 4). This may be due to the increase in number of cells in the hypertrophied glands caused by the initial goitrogenic effect and the subsequent removal of the block to iodine uptake resulting from the rapid decline in effectiveness of the potassium thiocyanate.

Discussion

In the present investigation, the uptake of radioactive iodine by the frog thyroid was ascertained after the injection of three antithyroid drugs (thiourea, sulfanilamide and potassium thiocyanate). From the foregoing results it is clear that thiourea, sulfanilamide and potassium thiocyanate, when injected into normal frogs in doses that are not in the usual sense to be considered toxic, induce a pronounced enlargement of the thyroid gland. These results are similar to those observed by Astwood, Sullivan, Bissell and Tyslowitz (1943) with thiourea and certain sulfonamides on the rat thyroid. Astwood (1943), VanderLaan and Bissell (1946b), and Wolff, Chaikoff, Taurog and Rubin (1946) obtained the same results with potassium thiocyanate. These drugs act primarily by blocking the utilization of iodine, and thyroid hyperplasia and hyperemia occur as secondary compensatory responses to the increased thyrotropic hormone output by the pituitary. The basic system governing the structure and function of the thyroid gland appears to be an interrelation and balance between the thyrotropic activity of the anterior pituitary and the production of a calorigenic substance from the thyroid gland.

By the use of the radioactive iodine technique, it was found that thiourea and sulfanilamide still exert a pronounced inhibitory effect on the thyroid twenty-four hours after injection, but this prolonged effect was not obtained with potassium thiocyanate. Collection of radioactive iodine by thyroids of potassium thiocyanate-treated frogs is similar to that observed for the rat thyroid by Franklin, Chaikoff and Lerner (1944). They observed that thyroid slices *in vitro* fail to absorb radioactive iodine in the presence of potassium thiocyanate. However, the thyroids of some potassium thiocyanate-treated frogs concentrated a greater per cent of the

injected labeled iodine than did their controls, but this effect was observed only when the time interval after the injection of the drug was prolonged to the point at which its disappearance from the circulation had occurred. The results of the present investigation show that potassium thiocyanate is rapidly removed from the circulation, but so long as a high concentration is present in the blood, the uptake of radioactive iodine by the frog·thyroid is definitely depressed. The inhibitory action of this drug was observed after a single injection of a 0.2 per cent solution of potassium thiocyanate, provided the labeled iodine is injected and the thyroids removed at a time when potassium thiocyanate is still present in the circulation.

The experiments reported above do not permit conclusions concerning all the details bringing about inhibition by these drugs, but it is assumed that iodine absorption is only one of the many steps in the manufacture of thyroid hormone which is in some way hindered. A more complete understanding of the processes controlling normal thyroid function would aid greatly in the interpretation of the whole mechanism of the goitrogenic effect of these compounds.

Summary

1. The iodine-concentration capacity of the thyroid gland, as measured by the uptake of radioactive iodine, was depressed in frogs injected with thiourea, sulfanilamide and potassium thiocyanate. The subcutaneous injection of 0.5 cc. of a 10 per cent solution of thiourea and of sulfanilamide, and 1 cc. of a 0.2 per cent solution of potassium thiocyanate into normal frogs resulted in an enlarged, hyperemic and hyperplastic thyroid gland. The thyroid hyperplasia which occurred under the influence of these drugs was a secondary compensatory response to the increased thyrotropic hormone output by the hypophysis. Inhibition was most pronounced with thiourea, sulfanilamide and potassium thiocyanate following in that order.

2. Injected potassium thiocyanate was rapidly removed from the blood stream, and its elimination was practically complete in 24 hours. However, the iodine-concentrating capacity of the thyroid gland, as measured by the uptake of radioactive iodine, was depressed by potassium thiocyanate, provided a high concentration of the drug was still present in the circulation. After 24 hours, the enlarged thyroids of the potassium thiocyanate-treated frogs had a greater capacity than normal for absorbing injected radioactive iodine.

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